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### **REMARKS**

Claims 1-3, 6-14, 18, and 20-22 are pending in the application. Claims 7-8 are cancelled. Claims 1, 3, 9, 14, 18 and 21 are currently amended. Claims 36-51 are currently added. The addition and amendment find support in the specification, e.g., on page 13, page 34, and page 60 of the substitute specification filed on April 8, 2002 to comply with margin requirement. Specifically, claim 3 and claim 14 amendment is supported on pages 3, 12-18. New claims 36-52 are supported on pages 3, 6, 12-18, 34 and 60. No new matter is added.

As a result of current amendment and addition, claims 1-3, 6, 9-14, 18, 20-22, and 36-51 are pending for examination.

#### **Claim 9**

Applicants confirm that claim 9 is currently pending for examination. The cancellation of claim 9 in our December 18, 2002 correspondence was due to clerical error. Claim 9 was acknowledged in the restriction requirement mailed by Patent Office on March 5, 2003, and was subsequently elected.

Applicants appreciate the consideration of claim 9 as currently amended by the Examiner.

#### **Election/Restriction**

Applicants submit that claims 1-3, 6-14, 18, 20-22 were elected for examination in our response filed April 4, 2003. Applicants request the proper examination of the pending claims including claim 18, which was omitted from the summary page of the present Office Action, but included in the detailed Office Action.

#### **Specification Objection**

Page 2 of the specification is objected to for reciting the Patent No. 6,008,205. Applicants submit that the clerical error in citing "6,008,205" has been corrected to "6,008,025". Applicants respectfully request the objection be withdrawn.

Claim Objection

Claim 7 is objected to for reciting “said first enzyme is Archaeal DNA polymerase.”

Claim 7 is cancelled.

Applicants respectfully request the withdrawn of the above objection on claim 7.

Claim rejections under 35 U.S.C. §112, Second Paragraph

Claims 1-3, 9-10, 11-14, 20-22 are rejected for indefiniteness under 35 U.S.C. §112, Second Paragraph.

Claims 1, 9, 12 and 21 and their dependent claims are rejected for reciting the phrase “one or more” because the Examiner states that the meaning of “*one or more*” is not clear. Applicants has amended claims 1, 9, 12 and 21 to recite “*said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.*” Therefore, the mutant Pfu DNA polymerase, as recited in the amended claims 1, 9, 12 and 21, includes a mutant Pfu DNA polymerase having a single mutation at any of the amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388. In addition, the mutant Pfu DNA polymerase, as recited in the amended claims 1, 9, 12, and 21, also include a mutant Pfu DNA polymerase having a mutation at *two or more (e.g., three, four, five, six, seven, eight or all nine) amino acid positions* selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

Based on the above amendment and clarification, Applicants believe that claims 1, 9, 12, as well as their dependent claims 2-3, 10-11, 13-14, 20 and 22, do particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Applicants respectfully request the withdrawal of rejections on claims 1-3, 9-10, 11-14, and 20-22 under 35 U.S.C. §112, second paragraph.

Claim Rejections under 35 U.S.C. §112, First Paragraph-Written Description

Claims 6-8, 18 and 20 are rejected under 35 U.S.C. §112, First Paragraph, for lack of written description. The Examiner states that the specification did not describe the subject matter of the above claims in a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had *possession* of the claimed invention. First, the Examiner points out where the claims refer to a mixture of *an Archaeal DNA polymerase and a mutant Archaeal DNA polymerase* selected from a number of different Archaeal DNA polymerases comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, the specification only provides an enzyme mixture comprises an Archaeal DNA polymerase and a mutant *Pfu* DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity wherein said mutant *Pfu* DNA polymerase comprises a mutation at D405, Y410, T542, D543, K593, Y595, Y385, G387, or G388. Second, the Examiner states that where the claims refer to a *Pfu* DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, the specification fails to describe additional representative species of these mutant enzymes by any identifying structural characteristics.

Applicants submit that claims 7-8 are cancelled. Applicants will continue the prosecution of subject matters in cancelled claims 7-8 in the copending application with Serial No. 10/079,241. Claims 6, 18, and 20 are amended, and new claims 36-51 are added.

With respect to the Examiner's first statement, Applicants submit that the currently pending claims are no longer drawn to an enzyme mixture comprising an Archaeal DNA polymerase and a mutant Archaeal DNA polymerase. Instead, claims 6, 18, and 20 are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant *Pfu* DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity. New independent claims, i.e., 36, 40, 44, and 48-50, are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant *Pfu* DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the

group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, where the first DNA polymerase is either Taq, KOD, or JDF-3 DNA polymerase.

Therefore, the Examiner's first statement does not apply to the currently pending claims, i.e., claims 1-3, 9-14, 18, 20-22, and 36-51.

With respect to the Examiner's second statement that the specification does not provide representative species of mutant enzymes comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity by any identifying structural characteristics, Applicants submit that new claims 36-51 do not recite a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity. Instead, claims 36-51 specifically point out that the second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388. Therefore, the Examiner's second statement does not apply to claims 36-51.

For claims 6, 18, and 20, Applicants submit that the specification does provide adequate description for an enzyme mixture comprising a first enzyme and a second enzyme, wherein said *first enzyme is either a wild type Pfu DNA polymerase or Taq DNA polymerase, a second enzyme which is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.*

MPEP 2163 provides:

"The written description requirement for a claimed genus may be satisfied through *sufficient description of a representative number of species by actual reduction to practice* (see i)(A), above), *reduction to drawings* (see i)(B), above), or *by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure*, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see i)(C), above). See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406."

The present specification provides functional description, e.g., by clearly defining 3'-5' exonuclease activity and DNA polymerization activity of a DNA polymerase including a Pfu DNA polymerase.

For example, the specification teaches on page 9, lines 3-13:

“As used herein, ‘exonuclease’ refers to an enzyme that cleaves bonds, preferably phosphodiester bonds, between nucleotides one at a time from the end of a DNA molecule.... For example, one unit of exonuclease activity may refer to the amount of enzyme required to cleave 1 µg DNA target in an hour at 37°C.”

The specification defines DNA polymerization activity as follows on page 11, lines 14-28:

“One unit of DNA polymerization activity of conventional DNA polymerase, according to the subject invention, is defined as the amount of enzyme which catalyzes the incorporation of 10 nmoles of total deoxynucleotides (dNTPs) into polymeric form in 30 minutes at optimal temperature (e.g., 72°C for Pfu DNA polymerase).”

The specification further teaches how to measure exonuclease activity and DNA polymerization activity in great details, e.g., on pages 27-30. Such assays are also known in the art, as described on page 11, line 28 to page 12, line 2 of the present specification:

“Assays for DNA polymerase activity and 3'-5' exonuclease activity can be found in DNA Replication 2nd Ed., Kornberg and Baker, supra; Enzymes, Dixon and Webb, Academic Press, San Diego, Calif. (1979), as well as other publications available to the person of ordinary skill in the art.”

In addition, the present specification provides detailed description on the structure-function relationship of Pfu DNA polymerase in view of its exonuclease activity and polymerization activity.

Pfu DNA polymerase is an Archaeal DNA polymerase known in the art, e.g., as described in Lundberg et al., 1991, *Gene*, 108:1 (page 11). Pfu DNA polymerase is also commercially available from Stratagene Inc. (La Jolla, CA) (page 11). The amino acid sequence Genbank accession number for Pfu DNA polymerase is known in the art (e.g., see page 15). The specification teaches that “[t]he DNA coding sequence of a wild-type Pfu DNA polymerase can

be found in the art, for example, from Genbank (accession No. U84155). A detailed description of the structure and function of Pfu DNA polymerase can be found, among other places in U.S. Patent Nos. 5,948,663; 5,866,395; 5,545,552; 5,556,772, all of which thereby incorporated by references. A not-limiting detailed procedure for preparing Pfu DNA polymerase with reduced polymerization activity is provided in Example 1.” (page 25, lines 7-12).

The specification provides description for polymerization domains and exonuclease domains (including 6 references) on page 19, lines 2-10:

“Polymerization and exonuclease domains (i.e., their crystal structures) of many DNA polymerases are known in the art (for examples, see Rodriguez et al., 2000, J. Mol. Biol. 299:447-62; Zhao et al., 1999, Structure Fold Des. 7:1189-99; Baker et al., 1998, Proc Natl Acad Sci U S A. 95:3507-12; Kiefer et al., 1997, Structure 5:95-108; Kim et al., 1995, Nature, 376:612-6; Kong et al., 1993, J Biol Chem. 268:1965-75).”

The specification teaches on page 20:

“With the great availability of sequences from DNA polymerases, it has become possible to delineate a few highly conserved regions for various polymerase types (for review, see for example, Johnson, 1993, Annu Rev Biochem. 62:685-713). Delarue et al. reported an approach for unifying the structure of DNA polymerase (1990, Protein Eng., 3:461-7). The speculative hypothesis should provide a useful model to direct genetic modifications for preparing DNA polymerase with reduced polymerization activity.” (lines 4-9)

The specification teaches that within the sequence of an  $\text{exo}^+$  DNA polymerase, e.g., a Pfu DNA polymerase, one preferred sequence for modification is the DNA sequence encoding the polymerization domain (page 19, lines 4-5).

The specification further teaches on page 19, lines 11-19:

“General structure features of DNA polymerization domain is known in the art. For example, Blanco et al. (1991, Gene, 100:27-38) discloses that *significant amino acid (aa) sequence similarity has been found in the C-terminal portion of 27 DNA-dependent DNA polymerases* belonging to the two main superfamilies: (i) Escherichia coli DNA polymerase I (PolI)-like prokaryotic DNA polymerases, and (ii) DNA polymerase alpha-like prokaryotic and eukaryotic (viral and cellular) DNA polymerases. The *six most conserved C-terminal regions*, spanning approximately 340 amino acids, are located in the same linear

arrangement and *contain highly conserved motifs and critical residues involved in the polymerization function.*”

The specification specifically provides examples of targeted amino acid residues within the above highly conserved polymerase domain motifs (DXXSLYP, KXXXNSXYG, TXXGR, YXDTDS, and KXY) and a partitioning domain YXGG motif that can be mutagenized to decrease DNA polymerase activity (page 21, lines 1-5 and page 21, lines 25-30).

Furthermore, the present specification provides sufficient description of a representative number of species by actual reduction to practice. It is established that a specification may, within the meaning of 35 U.S.C. §112, first paragraph, contain a written description of a claimed invention without describing all species that claim encompasses.

With respect to Pfu DNA polymerase specifically, the specification provides at least 9 amino acid positions for mutation (i.e., D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388), e.g., see page 25, lines 22-24. Specific mutations are provided for these positions, e.g., D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P, as disclosed on page 25, lines 26-27. Table 1 on pages 49-52 discloses 22 specific partitioning domain mutants and 7 specific polymerase domain mutants within 3 conserved polymerase domains (e.g., DXXSLYP motif containing D405 and Y410, YXDTDS motif containing T542 and D543, and KXY motif containing K593 and Y595).

In view of the above, Applicants submit that the level of skill in the art of DNA polymerase and recombinant DNA is high, that the present specification describes the function of a mutant Pfu DNA polymerase, specifically defines the structural features associated with such function, i.e., 3'-5' exonuclease activity and reduced DNA polymerization activity. One skilled in the art therefore can, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus.

Therefore, the present specification satisfies the written description requirement under 35 U.S.C. §112, First Paragraph. Applicants respectfully request the written description rejections be withdrawn.



Claim Rejections under 35 U.S.C. §112, First Paragraph-Enablement

Claims 6, 7, 8, 18, and 20 are rejected under 35 U.S.C. §112 for lack of enablement. The Examiner states that the specification does not provide enablement for the broad scope as claimed in claims 7, 18 and 20. The Examiner summarizes that the specification does not establish: (A) regions of the protein structure which may be modified without affecting 3'-exonuclease activity while causing a reduction in polymerizing activity; (B) the general tolerance of Archaeal DNA polymerases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any amino acid residue of any Archaeal DNA polymerase with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful. The Examiner concludes that the extended experimentation that would be required to determine which substitutions would be acceptable to retain the 3'-5' exonuclease activity while reducing or diminishing the polymerase activity, and the fact that the relationship between the sequence of a peptide and its tertiary structure (i.e., its activity) are not well understood and are not predictable, undue experimentation is required.

Applicants respectfully disagree.

As stated before, claims 7-8 are cancelled. Applicants will continue the prosecution of subject matters in cancelled claims 7-8 in the copending application with Serial No. 10/079,241. Claims 6, 18, and 20 are amended, and new claims 36-51 are added.

New independent claims, i.e., 36, 40, 44, and 48-50, are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations, wherein said mutation(s) are selected from amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388, where the first DNA polymerase is either Taq, KOD, or JDF-3 DNA polymerase.

Claims 6, 18, and 20 are drawn to an enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said

second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

In view of the above amendment, Applicants submit that the Examiner's statement (C) no longer applies, (A), (B), and (C) may apply with much narrower scope consistent with the scope of these claims in their current form.

MPEP 2164.01 provides the test of enablement:

"Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims *as to enable one skilled in the pertinent art to make and use the claimed invention*. The standard for determining whether the specification meets the enablement requirement was cast in the Supreme Court decision of *Mineral Separation v. Hyde*, 242 U.S. 261, 270 (1916) which postured *the question: is the experimentation needed to practice the invention undue or unreasonable?*"

As to how to make the claimed invention, MPEP 2164.01(b) provides:

"As long as the specification discloses *at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied*. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Failure to disclose other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. 112. *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir.), cert. denied, 484 U.S. 954 (1987).

...

*A key issue that can arise when determining whether the specification is enabling is whether the starting materials or apparatus necessary to make the invention are available...."*

As to the "how to use" requirement, MPEP 2164.01(c) provides:

"If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. 112 is satisfied...."

In contrast, when a compound or composition claim is not limited by a recited use, *any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use.* If multiple uses for claimed compounds or compositions are disclosed in the application, then an enablement rejection must include an explanation, sufficiently supported by the evidence, why the specification fails to enable each disclosed use. In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention.”

The Examiner’s enablement rejection appears to focus on the “how to make” requirement. Applicants submit that the present specification does satisfy the “how to make” requirement under 35 U.S.C. §112, first paragraph for enablement.

First, the level of skill in the art of DNA polymerase and modification is high.

In *Webster Loom v. Higgins*, 105 U.S. (15 Otto.) 580, 586 (1881), the Supreme Court emphasized that the specification need not be enabling to an “unskilled” layperson and may assume “that which is common and well known” to persons skilled in the relevant art.

Applicants submit that, although it is generally true that the relationship between the sequence of a peptide and its tertiary structure (i.e., its activity) are not well understood and are not predictable, that is not the case with DNA polymerases (e.g., Pfu DNA polymerase) as claimed in the claims of the present invention.

For example, the specification teaches:

“Polymerization and exonuclease domains (i.e., their crystal structures) of many DNA polymerases are known in the art (for examples, see Rodriguez et al., 2000, J. Mol. Biol. 299:447-62; Zhao et al., 1999, Structure Fold Des. 7:1189-99; Baker et al., 1998, Proc Natl Acad Sci U S A. 95:3507-12; Kiefer et al., 1997, Structure 5:95-108; Kim et al., 1995, Nature, 376:612-6; Kong et al., 1993, J Biol Chem. 268:1965-75).” (Page 19)

More specifically, the specification teaches that “[t]he DNA coding sequence of a wild-type Pfu DNA polymerase can be found in the art, for example, from Genbank (accession No. U84155). A detailed description of the structure and function of Pfu DNA polymerase can be found, among other places in U.S. Patent Nos. 5,948,663; 5,866,395; 5,545,552; 5,556,772, all of which thereby incorporated by references. A not-limiting detailed procedure for preparing Pfu

DNA polymerase with reduced polymerization activity is provided in Example 1.” (page 25, lines 7-12).

The specification teaches on page 20:

“With the great availability of sequences from DNA polymerases, it has become possible to delineate a few highly conserved regions for various polymerase types (for review, see for example, Johnson, 1993, *Annu Rev Biochem.* 62:685-713). Delarue et al. reported an approach for unifying the structure of DNA polymerase (1990, *Protein Eng.*, 3:461-7). The speculative hypothesis should provide a useful model to direct genetic modifications for preparing DNA polymerase with reduced polymerization activity.” (lines 4-9)

The pending claims are limited with the second enzyme being a mutant Pfu DNA polymerase.

As described above, Pfu DNA polymerase is an Archaeal DNA polymerase known in the art, e.g., as described in Lundberg et al., 1991, *Gene*, 108:1 (page 11). Pfu DNA polymerase is also commercially available from Stratagene Inc. (La Jolla, CA) (page 11). The amino acid sequence Genbank accession number for Pfu DNA polymerase is known in the art (e.g., see page 15). The specification also teaches that “[t]he DNA coding sequence of a wild-type Pfu DNA polymerase can be found in the art, for example, from Genbank (accession No. U84155). One skilled in the art, with the above teachings, can perform routine experiments to obtain a polynucleotide encoding Pfu DNA polymerase as the starting material for making the invention as claimed.

Second, the present specification discloses more than one method for making the invention as claimed. One skilled in the art can make the invention, i.e., a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity as claimed without undue experimentation.

A patent's specification need only provide one enabling method for making a claimed invention; that the specification may disclose other non-enabling methods is not necessarily fatal to the enablement of the invention (e.g., *Durel Corp. v. Osram Sylvania Inc.*, 256 F.3d 1298, 1307, 59 USPQ2d 1238, 1244 (Fed. Cir. 2001). Also see *Johns Hopkins Univ. v. CellPro, Inc.*,

152 F.3d 1342, 1361, 47 USPQ2d 1705, 1719 (Fed. Cir. 1998) (stating that the enablement requirement is met if the description enables any mode of making and using the invention).

The specification provides that mutant polymerases can be made by a number of methods, e.g., as described on pages 35. Specifically, the specification teaches how to perform genetic modification by either site-directed mutagenesis or random mutagenesis (e.g., pages 19-25); how to screen or examine the mutants for reduced DNA polymerization activity (e.g., pages 26-28); how to examine the mutants for 3'-5' exonuclease activity (e.g., pages 28-29); how to express and purify wild-type and mutant DNA polymerases (e.g., pages 30-32); and how to make a mixture of a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity (e.g., pages 32-36).

The specification teaches that within the sequence of an  $\text{exo}^+$  DNA polymerase, e.g., a Pfu DNA polymerase, one preferred sequence for modification is the DNA sequence encoding the polymerization domain (page 19, lines 4-5).

The specification further teaches on page 19, lines 11-19:

“General structure features of DNA polymerization domain is known in the art. For example, Blanco et al. (1991, Gene, 100:27-38) discloses that *significant amino acid (aa) sequence similarity has been found in the C-terminal portion of 27 DNA-dependent DNA polymerases* belonging to the two main superfamilies: (i) Escherichia coli DNA polymerase I (PolI)-like prokaryotic DNA polymerases, and (ii) DNA polymerase alpha-like prokaryotic and eukaryotic (viral and cellular) DNA polymerases. The *six most conserved C-terminal regions*, spanning approximately 340 amino acids, are located in the same linear arrangement and *contain highly conserved motifs and critical residues involved in the polymerization function.*”

The specification specifically provides examples of targeted amino acid residues within the above highly conserved polymerase domain motifs (DXXSLYP, KXXXNSXYG, TXXGR, YXDTDS, and KXY) and a partitioning domain YXGG motif that can be mutagenized to decrease DNA polymerase activity (page 21, lines 1-5 and page 21, lines 25-30).

Example 1 teaches constructing mutants of Pfu DNA polymerase with reduced DNA polymerase activity; example 2 teaches affinity purification of His-tagged Pfu DNA polymerase mutants; example 3 teaches assaying DNA polymerase and 3'-5' exonuclease activities of Pfu DNA polymerase mutants; example 4 teaches purification of Pfu DNA polymerase mutants by conventional column chromatography; and example 5 teaches verifying the presence of proofreading activity in Pfu mutants under PCR conditions.

Third, the specification provides a representative numbers of Pfu mutant by showing actual reduction to practice.

With a genus claim, e.g., claim 6, 18, 20 of the present invention, Applicants may satisfy the enablement requirement by showing the enablement of a representative number of species within the genus, this is analogous to written description of a genus under §112, first paragraph, see *Angstadt*, 537 F.2d at 502-03, 190 USPQ at 218.

With respect to Pfu DNA polymerase specifically, the specification provides at least 9 amino acid positions for mutation (i.e., D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388), e.g., see page 25, lines 22-24. Specific mutations are provided for these positions, e.g., D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P, as disclosed on page 25, lines 26-27. Table 1 on pages 49-52 discloses 22 specific partitioning domain mutants and 7 specific polymerase domain mutants within 3 conserved polymerase domains (e.g., DXXSLYP motif containing D405 and Y410, YXDTDS motif containing T542 and D543, and KXY motif containing K593 and Y595).

Applicants submit that the above teaching in the specification provides enablement of a representative number of Pfu mutant species within the genus of a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, as claimed in claims 6, 18, and 20, as well as claimed in the new claims 36-51.

Fourth, with respect to the Examiner's statement (A), Applicants submit that although in one embodiment, it is preferred that the 3'-5' exonuclease activity of the mutant DNA polymerase is not affected, i.e., still being equivalent to the level of the wild-type enzyme, this is

not a requirement recited in the claims. The specification specifically teaches how to check the 3'-5' exonuclease activity of a mutant DNA polymerase so as to make sure that the mutant DNA polymerase is one comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity, e.g., as described on pages 11-12 and example 3 of the present specification. One skilled in the art may perform such assay for a mutant DNA polymerase following routine laboratory procedure without undue experimentation.

The question of undue experimentation is a matter of degree. The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." *Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 U.S.P.Q. (BNA) 409, 413 (Fed. Cir. 1984).

The Patent and Trademark Office Board of Appeals summarized the point well when it stated:

The test is not merely quantitative, since a considerable amount of experimentation is permissible, *if it is merely routine*, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. (*Ex parte Jackson*, 217 U.S.P.Q. (BNA) 804, 807 (1982)).

Applicants submit that, once a DNA polymerase with reduced polymerization activity is made according to the teachings of the present specification, a person of ordinary skills in the pertinent art would only have to perform routine experiments known in the art, e.g., exonuclease assay as known in the art and taught in the present specification to examine such mutant to make sure that it also has 3'-5' exonuclease activity.

Fifth, the Examiner's position with respect to multiple substitutions (statement B) appears to be that the claims include inoperative embodiments. The Examiner is directed to *Atlas power Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

“Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. ‘It is not a function of the claims to specifically exclude . . . possible inoperative substances . . . .’ In re Dinh-Nguyen, 492 F.2d 856, 858-59, 181 U.S.P.Q. (BNA) 46, 48 (CCPA 1974) (emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 U.S.P.Q. (BNA) 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 U.S.P.Q. (BNA) 331, 334-35 (CCPA 1973). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid. See, e.g., In re Cook, 58 C.C.P.A. 1049, 439 F.2d 730, 735, 169 U.S.P.Q. (BNA) 298, 302 (1971).”

As discussed above, the skill in the art of DNA polymerases and their modification (e.g., by recombinant DNA techniques) is high. A person skilled in the art can produce DNA polymerases with the claimed multiple substitutions given the benefit of the present teaching. A person skilled in the art can also figure out which combination of multiple substitutions does not produce a mutant Pfu DNA polymerase comprising a 3’-5’ exonuclease activity and a reduced DNA polymerization activity. None of these requires undue experimentation of such person with ordinary skills in the art.

In view of the above, Applicants submit that the pending claims 1-3, 6, 9-14, 18, 20-22, and 36-51 satisfy the enablement requirement under 35 U.S.C. §112, first paragraph. Applicants respectfully request the enablement rejections on claims 6, 18 and 20 be withdrawn.

Claims 3, 8, and 14 are also rejected under 35 U.S.C. §112 for lack of enablement. The Examiner states that the polymerases, namely, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase, are not fully disclosed or been shown to be publicly known and freely available. The Examiner states that a deposit of these polymerases or bacteria should have been made in accordance with 37 CFR 1.90101.809.

Claim 8 is cancelled.

MPEP 2411.01 provides:

“(A) 35 U.S.C. 112, first paragraph - lack of an enabling disclosure without access to a specific biological material. *This ground of rejection should be*



*accompanied by evidence of scientific reasoning to support the conclusion that a person skilled in the art could not make or use the invention defined in and commensurate with the claims without access to the specific biological material.”*

Applicants submit that a deposit is not required under the circumstances being considered because the DNA polymerases claimed are known in the art.

The specification provides accession number for each of the claimed DNA polymerase, each protein accession number is accompanied with the corresponding DNA sequence accession number, as well as the corresponding reference. These DNA polymerases are published and publicly known in the art.

In addition, the specification teaches examples of the generally known polymerases and gives references for each on page 11:

“Known conventional DNA polymerases include, for example, ***Pyrococcus furiosus* (Pfu) DNA polymerase** (Lundberg et al., 1991, *Gene*, 108:1, provided by Stratagene), ***Pyrococcus woesei* (Pwo) DNA polymerase** (Hinnisdaels et al., 1996, *Biotechniques*, 20:186-8, provided by Boehringer Mannheim), ***Thermus thermophilus* (Tth) DNA polymerase** (Myers and Gelfand 1991, *Biochemistry* 30:7661), ***Bacillus stearothermophilus* DNA polymerase** (Stenesh and McGowan, 1977, *Biochim Biophys Acta* 475:32), ***Thermococcus litoralis* (Tli) DNA polymerase** (also referred to as Vent DNA polymerase, Cariello et al., 1991, *Polynucleotides Res*, 19: 4193, provided by New England Biolabs), 9°Nm DNA polymerase (discontinued product from New England Biolabs), ***Thermotoga maritima* (Tma) DNA polymerase** (Diaz and Sabino, 1998 *Braz J. Med. Res*, 31:1239), ***Thermus aquaticus* (Taq) DNA polymerase** (Chien et al., 1976, *J. Bacteriol*, 127: 1550), ***Pyrococcus kodakaraensis* KOD DNA polymerase** (Takagi et al., 1997, *Appl. Environ. Microbiol.* 63:4504), **JDF-3 DNA polymerase** (from *thermococcus* sp. JDF-3, Patent application WO 0132887), ***Pyrococcus GB-D* (PGB-D) DNA polymerase** (also referred as Deep-Vent DNA polymerase, Juncosa-Ginesta et al., 1994, *Biotechniques*, 16:820, provided by New England Biolabs), **UITma DNA polymerase** (from *thermophile Thermotoga maritima*; Diaz and Sabino, 1998 *Braz J. Med. Res*, 31:1239; provided by PE Applied Biosystems), **Tgo DNA polymerase** (from *thermococcus gorgonarius*, provided by Roche Molecular Biochemicals), ***E. coli* DNA polymerase I** (Lecomte and Doubleday, 1983, *Polynucleotides Res.* 11:7505), **T7 DNA polymerase** (Nordstrom et al., 1981, *J. Biol. Chem.* 256:3112), and archaeal **DP1/DP2 DNA polymerase II** (Cann et al., 1998, *Proc Natl Acad Sci U S A* 95:14250-5). The polymerization activity of any of the above enzymes can be defined by means well known in the art.” (Emphasis added)

Therefore, deposits for the claimed DNA polymerases are not required because one skilled in the art could make or use the invention defined in and commensurate with the claims without access to the specific biological material. Applicants respectively request the withdrawn of the enablement rejection on claims 3 and 14.

In view of all of the above, Applicants submit that all pending claims, including the new claims 36-51, satisfy the enablement requirement. Applicants respectfully request the withdrawn of the enablement rejections on claims 3, 6, 14, 18, 20 and their dependent claims.

Claim Rejections under 35 U.S.C. §103

Claims 1-3, 7, 8, 10-14, 18, 20-22 are rejected as being obvious over Barnes et al., (U.S. Patent No. 5,436,149 and Komori et al.

The Examiner states that, specifically, Barnes teach a formation comprising at least one thermostable DNA polymerase lacking 3'-5' exonuclease activity and at least one thermostable DNA polymerase exhibiting 3'-5' exonuclease activity, where the thermostable DNA polymerase exhibiting 3'-5' exonuclease activity is a variant of the Pfu DNA polymerase *"wherein the DNA polymerase activity of said Pfu DNA polymerase has been diminished or inactivated."* The Examiner states that "Komori et al. teach the functional interdependence of DNA polymerizing and 3'-5' exonucleolytic activities in Pfu DNA polymerase...a number of Pfu DNA polymerase mutants which affect both the DNA polymerizing and/or the 3'-5' exonucleolytic activity in varying amounts." The Examiner gives two specific Pfu mutants taught in Komori et al., i.e., D405A and D405E. The Examiner states that one skilled in the art would have been motivated to used the mutants taught in Komori et al. in the formulation disclosed in Barnes et al. because Barnes teach "that the ratio of the 'polymerase without 3'-exonucleolytic activity' to the 'polymerase with 3'-exonucleolytic activity, wherein the polymerase activity is reduced or diminished' is high."

Applicants respectfully disagree.

Claims 7-8 are cancelled.

Applicants submit that it is essential for the second DNA polymerase of the present invention to have a reduced DNA polymerase activity as required in the claims 1-3, 10-14, 18, and 20-22.

The present specification teaches specifically about the advantage of reducing DNA polymerization activity:

“In addition to providing high fidelity for DNA synthesis, the compositions of the subject invention *prevent side effects of a high polymerization activity, therefore, increase the efficiency of the amplification compared to a mixture in which both DNA polymerases possess wild-type polymerization activities.*” (page 7, lines 19-22)

“*In order to prevent the side effects of having a high DNA polymerization activity in an amplification reaction,* the polymerization activity of the composition of the invention may also be reduced by physical and/or chemical modification and/or inhibition.” (page 31, lines 15-17)

Neither Komori et al. or Barnes et al. teach or suggest the importance of having a reduced DNA polymerization activity.

Komori et al. describe research investigating the structure-function relationship of Pfu DNA polymerase. Komori et al. report two substitution Pfu mutants with reduced DNA polymerase activity to establish the importance of the substituted residue in maintaining the polymerase activity of Pfu DNA polymerase. Komori et al. do not teach an enzyme mixture comprising a first and a second enzymes, wherein the second enzyme is a mutant Pfu DNA polymerase. In fact, Komori et al. do not teach or suggest any utility for the Pfu mutants with reduced DNA polymerase activity, e.g., for PCR amplification.

Barnes et al. teach a formulation of thermostable DNA polymerases comprising at least *one thermostable DNA polymerase lacking 3'-exonuclease activity (exo<sup>-</sup>)* and at least *one thermostable DNA polymerase exhibiting 3'-exonuclease activity (exo<sup>+</sup>)*. The formulation is claimed based on the hypothesis that the use of a DNA polymerase exhibiting *3'-exonuclease activity* can overcome the problem of a DNA polymerase lacking *3'-exonuclease activity*, e.g., Taq DNA polymerase. Barnes et al. provides:

“As speculated in Barnes (1992; supra), *Thermus aquaticus* DNA polymerase and its variants are slow to extend a mismatched base pair (which they cannot remove since they lack any 3'-exonuclease. A couple of companies (New England Biolabs and Stratagene) have introduced thermostable enzymes which exhibit a 3'-(editing) exonuclease which should, one would think, allow the removal of mismatched bases to result in both efficient extension and more accurately copied products. In practice, these two enzymes (Vent and Pfu DNA polymerase) are unreliable and much less efficient than expected....

I have discovered that the expected beneficial effects of a 3'-exonuclease can be obtained with an unexpectedly minute presence of an Archaeobacterial DNA polymerase, whilst *efficient extension is being catalyzed by a large amount of (3'-exonuclease-free) KlenTaq-278 or AT.*” (Columns 16-17).

As one can see, Barnes specifically teaches a combination of *an  $exo^-$  and an  $exo^+$  DNA polymerases* so that the amplification efficiency of  $exo^-$  may be increased in the presence of the  $exo^+$  DNA polymerase. For this purpose, we believe, and the Examiner correctly points out, that “*the only functional property of the second polymerase that is important is the presence of the 3' exonucleolytic activity*” (page 13 of the Office Action).

Barnes et al. mention only in the claims that the  $exo^+$  DNA polymerase is selected from the group consisting of Pfu polymerase from *Pyrococcus furiosus*, the Vent DNA polymerase from *Thermococcus litoralis*, a variant of the Pfu DNA polymerase wherein *the DNA polymerase activity of said Pfu DNA polymerase has been diminished or inactivated*, or a variant of the Vent DNA polymerase wherein the DNA polymerase activity of said Vent DNA polymerase has been diminished or inactivated.

The Pfu DNA polymerase mutant with *diminished or inactivated DNA polymerase activity* is one of the options for the thermostable DNA polymerase exhibiting 3'-exonuclease activity according to Barnes et al. Barnes et al., however, does not provide any teaching as to why a Pfu DNA polymerase mutant with *diminished or inactivated DNA polymerase activity* is desired, nor does it teach whether the use of a Pfu DNA polymerase mutant with *diminished or inactivated DNA polymerase activity* is advantageous over the use of a wild-type Pfu DNA polymerase.

One skilled in the art, based on the teaching of Barnes et al., in the absence of the present teaching, would not have been motivated to make an enzyme mixture comprising a first and second enzymes, wherein the second enzyme is a mutant DNA polymerase comprising a reduced DNA polymerase because Barnes does not teach why such mutant should be chosen over the other wild-type DNA polymerase options provided by Barnes et al.

In addition, for claims 6, 9, 18, 21, 40, 44, 49-50 and their dependent claims, the first enzyme is limited to an  $\text{exo}^+$  DNA polymerases, e.g., Pfu, KOD or JDF-3 DNA polymerase. Barnes et al. do not teach or suggest a combination of an  $\text{exo}^+$  DNA polymerase with another  $\text{exo}^+$  DNA polymerase with reduced polymerization activity as claimed in these claims. Komori et al. do not teach an enzyme mixture at all.

In view of the above, Applicants submit that Neither Barnes et al, or Komori et al., alone or in combination, teach the invention as claimed in the pending claims of the present invention, i.e., to make an enzyme mixture comprising a first enzyme and a second enzyme, where the second enzyme is required to have a reduced polymerization activity. There is no motivation for one skilled in the art to come to the invention as claimed in the claims, absent the benefit of the present teaching.

Applicants respectfully request the obviousness rejections on claims 1-3, 10-14, 18, 20-22 been withdrawn.

#### Claim Rejections under 35 U.S.C. 101 Double Patenting

Claims 1-3, 6, 9-14, 18, and 20-22 are provisionally rejected for statutory type double patenting in view of claims 1-3, 6, 9-11, 13-15, 19, and 21-23 of copending application with Serial No. 10/079,241.

Applicants submit that the copending application 10/079,241 is a continuation-in-part of the present application. Applicants have cancelled claims 1-3, 6, 9-11, 13-15, 19, and 21-23 of the copending application and claims 7-8 in the present application. As the result of the claim cancellation in the two applications, the invention as claimed in claims 1-3, 6, 9-14, 18, and 20-22 of the present application is being prosecuted in the present application, while invention as

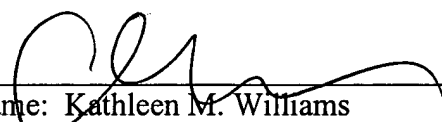
claimed in claims 7-8 of the present application are being prosecuted in the copending application.

In view of the above claim amendment in the present and the copending applications, Applicants respectfully request the double patenting rejection being withdrawn. No additional search is required for the Examiner to continue the examination of inventions of both patent applications as represented by their currently pending claims.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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